

Sequence and Functional Analysis of the Human Adenovirus Type 7 E3-gp19K Protein from 17 Clinical Isolates

TERRY W. HERMISTON,* ROBB HELLWIG,* JOHN C. HIERHOLZER,† AND WILLIAM S. M. WOLD*¹

*Department of Molecular Microbiology and Immunology, St. Louis University School of Medicine, 1402 South Grand Boulevard, St. Louis, Missouri 63104; and †Center for Disease Control, Respiratory and Enteric Viruses Branch, Division of Viral Diseases, 1600 Clifton Road, Atlanta, Georgia 30333

Received May 24, 1993; accepted August 25, 1993

The adenovirus (Ad) early region 3 (E3) glycoprotein of 19K (gp19K) binds major histocompatibility (MHC) class I antigens in the endoplasmic reticulum (ER), and the gp19K-class I complex is retained in the ER through an ER retention signal at the C-terminus of gp19K. This retention of class I antigens blocks cytolysis of gp19K-expressing cells by cytotoxic T lymphocytes (CTL). Animal models infected with Ad mutants lacking gp19K support a role for gp19K in counteracting a CTL response. Gp19K binds with different avidities to different class I antigens, and portions of the gp19K sequence are highly variable among Ad serotypes in different subgroups (Ad3, 11, and Ad35 in subgroup B; Ad2 and Ad5 in subgroup C); this raises the possibility that certain human individuals may be more susceptible to productive or persistent infection by particular serotypes of Ad, depending on the haplotype of the individual and the type of Ad. To begin to address this possibility, the gp19K gene from 17 very diverse Ad7 (subgroup B) clinical isolates was amplified by the polymerase chain reaction, and the DNA sequences were determined. The Ad7 gp19K sequence was 98% identical to that of Ad3. Surprisingly, we found complete conservation of the amino acid sequence of gp19K from all but one of the clinical isolates; one isolate had a conservative Ala to Val substitution. Gp19K from Ad7 clinical isolates representing distinct Ad7 genotypes co-immunoprecipitated with class I antigens. Our data indicate that there is very strong evolutionary pressure to maintain the sequence of gp19K in Ad7. The only known function for gp19K from different Ad serotypes is binding to class I antigens. It is interesting to consider, therefore, what selective pressure operates to maintain the sequence of gp19K among serotypes within a subgroup, and yet allows for very significant divergence in the sequence of gp19K among serotypes in different subgroups. The possible role of MHC class I antigens in this selection process is discussed. © 1993 Academic Press, Inc.

INTRODUCTION

Human adenoviruses (Ad) cause a variety of diseases including respiratory, gastrointestinal, urinary, and ocular infections, and they may be shed for years postinfection in the normal individual (Fox *et al.*, 1977; Hierholzer, 1992; Horwitz, 1990; Wigand and Adrian, 1986). The E3 region of Ad is thought to play a role in viral persistence and pathology, inasmuch as E3 encodes a variety of immunoregulatory proteins (Gooding, 1992; Wold and Gooding, 1991). To date, four immunoregulatory proteins from E3 have been described. Three of these proteins, termed 14.7K (14,700 MW), 14.5K, and 10.4K, prevent cytolysis of Ad-infected cultured cells by tumor necrosis factor (Gooding *et al.*, 1990; Gooding *et al.*, 1988; Gooding *et al.*, 1991). The fourth E3 protein is an abundant 19K glycoprotein (termed E3-gp19K or E3/19K) which binds class I antigens of the major histocompatibility complex (MHC).

Complex formation between MHC class I molecules and gp19K occurs in the endoplasmic reticulum (ER),

where the gp19K-class I complex is retained through an ER retention signal located at the C-terminus of gp19K (Andersson *et al.*, 1985; Burgert and Kvist, 1985; Cox *et al.*, 1991; Gabathuler and Kvist, 1990; Jackson *et al.*, 1990; Pääbo *et al.*, 1987). The binding of gp19K to class I molecules does not require other Ad proteins (Gabathuler *et al.*, 1990; Pääbo *et al.*, 1986a; Rawle *et al.*, 1989), and it occurs with class I antigens of rat, mouse, monkey, and human (Flomenberg *et al.*, 1992; Kvist *et al.*, 1978; Signäs *et al.*, 1986).

Viral peptides complexed with class I molecules on the cell surface serve as a signal for virus-specific cytotoxic T lymphocytes (CTL) to lyse the infected cell. By retaining class I molecules in the ER, gp19K should protect cells from lysis by Ad-specific CTL. Consistent with this, Rawle *et al.* (1989), using a mouse model system, showed that Ad-specific CTLs efficiently lysed cultured cells infected by Ad mutants lacking gp19K and that the presence of gp19K afforded protection.

There is evidence that gp19K may counteract immunosurveillance *in vivo*. Human Ad infection can induce a pneumonia in the cotton rat, *Sigmodon hispidus*, that is similar to that of the human (Pacini *et al.*, 1984). In the cotton rat, Ad mutants lacking gp19K showed

¹ To whom reprint requests should be addressed.

exaggerated pathogenicity over the wild-type virus, marked by enhanced lymphocyte and monocyte/macrophage infiltration (Ginsberg *et al.*, 1989). This may imply that the loss of gp19K allowed for MHC class I cell surface expression and enhanced the inflammatory response to the virally infected cells (Ginsberg *et al.*, 1989).

The 47 serotypes of human Ads form six subgroups, A to F, based on a variety of properties including quite divergent DNA genome sequences (Gooding and Wold, 1990; Green *et al.*, 1979; Hierholzer, 1992; Wiggand and Adrian, 1986). Pääbo *et al.* (1986b), using the W6/32 monoclonal antibody specific to the HLA-A, B, and C class I antigens, provided evidence that gp19K exists in Ad3, Ad11, Ad34 (subgroup B), Ad2, Ad5 (C), Ad9, Ad19 (D), and Ad4 (E), and that the gp19K from these serotypes can bind class I antigens. The gp19K gene has been sequenced in the subgroup B serotypes Ad3 (Signäs *et al.*, 1986), Ad11 (Mei and Wadell, 1992), and Ad35 (Flomenberg *et al.*, 1988), and the Ad35 gp19K protein was shown to bind class I antigens (Flomenberg *et al.*, 1987). No evidence for gp19K was obtained for Ad12 or Ad31 (subgroup A) (Pääbo *et al.*, 1986b), but the E1A gene of Ad12 inhibits synthesis of mRNA for class I antigens (Bernards *et al.*, 1983). Thus, down-regulation of cell-surface class I antigen expression, either by gp19K or by group A Ad E1A, is likely to be important in Ad pathogenesis in humans.

It is clear that the Ad2 or Ad5 gp19K proteins differ in their ability to form a complex with different MHC class I antigens. In the murine system, gp19K complexes well with K^d, L^d, and D^b, modestly with D^d, and not at all with D^k, K^k, and K^b (Burgert and Kvist, 1987; Cox *et al.*, 1990; Rawle *et al.*, 1989; Tanaka and Tevethia, 1988). Much less is known about the differential affinity of human class I antigens for gp19K: in the only published study, HLA-A2 was found to bind about twice as well to gp19K of Ad2 than did HLA-B7 (Severinsson *et al.*, 1986). Ad2 gp19K also binds to HLA-B27 (Gabathuler *et al.*, 1990). How this correlates with disease is not clear, but it suggests that the particular class I antigen and its affinity for a particular gp19K protein may be a major determinant in viral infection and subsequent pathogenesis.

Ad7, a member of subgroup B, commonly infects the respiratory tract of adults and children, causing severe pneumonia, acute respiratory disease in military recruits, pharyngoconjunctival fever, and keratoconjunctivitis. Other tissues can also be infected, and the infections can range from mild to severe or even fatal (Horwitz, 1990). Restriction endonuclease digestion studies on DNA from Ad7 (Adrian *et al.*, 1989b; Li and Wadell, 1986; Wadell, 1984) and Ad3 (Adrian *et al.*, 1989a; Li and Wadell, 1988; O'Donnell *et al.*, 1986; Wadell, 1984) have detected strains with different cleavage patterns. These "molecular epidemiology"

studies have been useful in correlating genotypes with clusters of infection and with geographical location, but they have not provided insight into the genotypic basis for the pathogenicity of the clinical isolates.

One model for Ad pathogenesis holds that differences in gp19K binding to class I antigens may account for differences in the severity or extent of infection. An alternate model would be that host factors (and perhaps other Ad genes) may be more important determinants in Ad infections. We begin to address these models by examining gp19K from 17 different Ad7 clinical isolates. This is the first study in which the sequence of a single Ad gene has been determined for different clinical isolates.

MATERIALS AND METHODS

Cells and viruses

Human A549 cells (lung carcinoma, obtained from ATCC, Rockville, MD) and human 293 cells (Graham *et al.*, 1977; obtained from ATCC) were maintained in Dubecco modified minimal essential medium containing 10% fetal calf serum.

Virus stocks of Ad3 and Ad5 were grown in suspension cultures of human KB cells, banded in CsCl, and titered on A549 cells as described (Green and Wold, 1979).

The Ad7 strains were originally isolated from eye, throat, and rectal specimens between 1954 and 1985 (see Table 1). Specimens were processed with antibiotics and inoculated onto human laryngeal epidermoid carcinoma (HEp-2) and primary human embryonic kidney (HEK) cells for recovery of virus, and the isolates were then serotyped by neutralization and hemagglutination-inhibition tests as described (Hierholzer, 1992). The passage level at this point was four to six passages in HEp-2 or HEK cells. After shipment to St. Louis, each virus isolate was propagated once on a 35-mm dish of 293 cells. After the appearance of a 100% cytopathic effect, the cells were suspended in the medium and lysed by freeze-thawing three times. After centrifugation to remove the cellular debris, the virus titer was determined on A549 cells.

Polymerase chain reaction (PCR)

Viral templates used for PCR were prepared by adding equal volumes of viral supernatant (described in the previous section) and 2X proteinase K buffer [200 mM Tris-Cl (pH 8.0), 300 mM NaCl, 25 mM EDTA, 2% sodium dodecyl sulfate (SDS), and proteinase K to a final concentration of 500 µg/ml (Sigma Chemical Co., St. Louis, MO)] and incubating at 37° for 1 hr. The sample was extracted with phenol:chloroform, chloroform and ethanol precipitated.

Two oligonucleotides (Operon Technologies, Alameda, CA), a 5'-end primer containing a *Bam*HI site, 5'-GGT CGT GGG ATC CCC ATG GGG GCC ATA TTA GTC CTG-3', and a 3'-end primer containing an *Eco*RI site, 5'-CAG TCA CGA TGA ATT CCC TTA AGG CAT TTT TTC TTT TTC C-3', were used to prime the PCR reaction. The sequences used for the primers were obtained from A. Davis and P. Hung (personal communication). The PCR reaction was performed at St. Louis University on a Perkin Elmer Cetus thermocycler (Norwalk, CT) using 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μ M dNTPs, 1 μ M for each primer, and 2.5 units of Taq DNA polymerase (Promega), and under melting conditions of 94° for 1 min, annealing at 55° for 1 min, and extension at 72° for 2 min, which was repeated for 30 cycles. The reaction was repeated in Atlanta with an initial melting step of 94° for 10 min, an annealing step of 62° for 20 sec, and an extension step of 74° for 1 min. After the initial round of synthesis, the melting conditions were changed to 94° for 45 sec and the cycle was repeated 30 times. In both cases, a DNA-negative control tube was included to detect viral DNA carry over or contamination.

Individual PCR products were gel-purified using 1.5% agarose gels, collected on Schleicher and Schuell NA45 paper (Keene, NH), and digested with restriction endonucleases *Bam*HI and *Eco*RI (Promega Corp., Madison, WI). These fragments were ligated to the vector plasmid pGEX-3X (Pharmacia, LKB Biotechnology, Inc., Piscataway, NY) which had been digested with *Bam*HI and *Eco*RI and treated with calf intestinal phosphatase (Promega, as described by the manufacturer). Usually three, and at least two different individual plasmid isolates were pooled for sequencing in an attempt to eliminate artifacts introduced through misincorporation by Taq polymerase.

DNA sequencing was performed by the Sanger method using the two PCR oligonucleotides mentioned previously and two additional internal primers, 5'-ATG GGA GCC AGG AGT TCC CG-3' and 5'-ATG GAA CCG TCA GGA CCT CG-3', in order to completely sequence both strands of the Ad7 gp19K-specific DNA. The U.S. Biochemical Co. kit (Cleveland, OH) was used for sequencing.

Metabolic labeling of proteins, immunoprecipitation, and SDS-polyacrylamide gel electrophoresis

A549 cells were mock-infected or infected with 50 PFU per cell of virus, were labeled with 100 μ Ci of *Tran*³⁵S-label (>1000 Ci/mmol; ICN Biomedical, Inc., Costa Mesa, CA) per milliliter from 8 to 12 hr postinfection. Proteins were extracted, immunoprecipitation was carried out using the W6/32 monoclonal antibody (ATCC) to human class I antigens, and the samples

were analyzed by SDS-PAGE as described by Hermiston *et al.* (1993).

RESULTS

The Ad7 isolates examined were collected from throughout the world (continental United States, Spain, Peru, New Guinea), in different years, from different tissues, from patients with different disease severity (death to mild infection and presentation), and from patients of different age and sex (Table 1). The isolates include representatives of Ad7 genome types D1 through D7 as well as D9; these genome types are based on restriction endonuclease polymorphisms (Adrian *et al.*, 1989b). Each isolate was propagated once on a dish of human 293 cells, and the gp19K gene was amplified by PCR, cloned, and sequenced. The PCR product was of the size expected from the sequence obtained and the primers used (data not shown).

Figure 1 shows the sequence of the Ad7 gp19K gene and protein from the prototype Ad7 isolate, Gomen. The sequence of gp19K is very similar to that of other subgroup B serotypes, Ad3, Ad11, and Ad35 (Fig. 2A), but it is quite diverged from gp19K of Ad2 (Fig. 2C). Ad2 gp19K has a cleaved N-terminal signal sequence (Kämpe *et al.*, 1983; Wold *et al.*, 1985), two Asn-linked glycosylation sites (Kornfeld and Wold, 1981; Wold *et al.*, 1985), a transmembrane domain near the C-terminus (Hérisse *et al.*, 1980), and a polar cytoplasmic domain that stops translocation through the membrane of the ER and retains the protein in the ER (Gabathuler and Kvist, 1990; Jackson *et al.*, 1990; Pääbo *et al.*, 1987). These features all appear to be conserved in the subgroup B versions of gp19K, except the subgroup B proteins have four potential Asn-linked glycosylation sites, at least some of which are glycosylated (Flomenberg *et al.*, 1988). Remarkably, there are only three differences between the Ad7 and Ad3 versions of gp19K (Fig. 2A), and one of these is in the putative cleaved N-terminal signal sequence and another is in the cytoplasmic domain.

When the gp19K gene from the 17 clinical isolates was sequenced, surprisingly, only five nucleotide changes were detected (see the legend to Fig. 1). In all but one case, the nucleotide change made no difference in the amino acid codon. With isolate 722, the nucleotide change caused an Ala to Val substitution at position 42 in the complete sequence (Fig. 1), which is equivalent to position 23 following cleavage of the signal sequence at the predicted site (Fig. 2A). To exclude the possibility that we had amplified the same DNA sample repeatedly, four samples (Gomen, 641, 722, and V-1887-I) were amplified by PCR in Atlanta and then sent to St. Louis for sequencing. The same sequence was obtained for both the Atlanta and St. Louis

TABLE 1
DESCRIPTION OF Ad7 CLINICAL ISOLATES

Genome type ^a	Strain	Origin	Year	Illness ^b	Pathogenicity	Age	Sex	Source ^c
D1	Gomen	California	1954	URI	Mod.	?	M	T
D1	641	Colorado	1972	PCF	Mild	28	M	E
D1	V-1755	Seattle	1984	CNS, pneum	Severe	4	F	R
D2	S 1058	Maryland	1958	URI	Mod.	?	?	T
D2	104	Georgia	1965	pneum, gen	Fatal	1	F	H
D2	441	Georgia	1971	PCF	Mild	6	M	T
D3	18	Maine	1961	CNS, pneum	Severe	14	M	R
D3	722	Peru	1972	CNS, URI, GI	Fatal	5	M	R
D4	603	Spain	1969	URI	Severe	23	M	T
D4	V-1887-I	New Guinea	1983	PCF	Mild	19	M	N
D5	375	New York	1970	PCF	Mod.	30	M	E
D5	V-1887-A	New Guinea	1983	Pneum, OM	Severe	14	M	N
D5	V-1803	South Dakota	1985	Cough	Mild	1	M	N
D6	945	Texas	1974	Influenza	Mod.	10	F	T
D7	1184	Massachusetts	1976	Influenza, URI	Mod.	20	F	R
D9	1267	Georgia	1977	PCF	Mod.	6	M	E
D9	1270	Georgia	1977	Influenza, GI	Severe	47	M	E

^a D1 through D9 refers to different genotypes as determined by restriction endonuclease polymorphisms (Adrian *et al.*, 1989b).

^b URI, upper respiratory infection, including tonsillitis, pharyngitis, rhinitis, and fever; PCF, pharyngoconjunctival fever; CNS, central nervous system infection; GI, gastrointestinal tract infection with diarrhea or gastroenteritis; OM, otitis media; pneum, pneumonia; gen, generalized illness.

^c Source of isolate: E, eye swab; H, autopsy heart; N, nasal wash; R, rectal; T, throat.

PCR products, indicating that the sequences obtained were correct.

Metabolic radiolabeling and class I-gp19K coimmunoprecipitation assays were carried out to confirm that the gp19K protein is expressed and functional in the clinical isolates. A549 cells were infected with an Ad7 clinical isolate representative of each genomic type (D1-D7, D9) as designated in Table 1. MHC class I monoclonal antibody W6/32 was used for the coimmunoprecipitation assay; this antibody reacts with all human class I antigens. Ad5, a subgroup C Ad whose gp19K differs significantly in amino acid sequences from gp19K of the subgroup B isolates, was included as a positive control because the binding of class I antigens to gp19K of subgroup C Ads has been extensively studied (see Hermiston *et al.*, 1993). Ad3, whose gp19K is very similar to that of Ad7 (Fig. 2A), was also included. As shown in Fig. 3, all the Ad7 isolates tested produced a functional gp19K protein, as identified by coimmunoprecipitation with class I antigens. β_2 -Microglobulin was also coimmunoprecipitated. The migration patterns of gp19K from the different clinical isolates were identical and comigrated with the subgroup B Ad3 gp19K protein, in contrast to the subgroup C Ad5 gp19K protein which migrated further in the gel. This supports the sequencing data and confirms that the gp19K proteins of these clinical isolates are expressed and functional.

The subgroup C version of gp19K migrates in SDS-PAGE with an apparent MW of about 25K when it is

Asn-glycosylated with exclusively high-mannose oligosaccharides at both its potential sites, but only at about 19K when it is not glycosylated (Kornfeld and Wold, 1981; Wold *et al.*, 1985). The Ad7 and Ad35 versions of gp19K have been shown previously to be glycosylated with exclusively high-mannose oligosaccharides, probably at all four potential sites, and to migrate in SDS-PAGE at about 29K (Flomenberg *et al.*, 1987; Kapoor *et al.*, 1981). Gp19K from Ad3 and the Ad7 clinical isolates also migrates at about 28K in the gel in Fig. 3, indicating that the proteins are glycosylated.

DISCUSSION

One of the questions that we have asked is whether clinical manifestation of Ad7 isolates is determined by differences in the sequence of the well-described Ad immunoregulatory protein, E3-gp19K. Using 17 different Ad7 clinical isolates, which include eight of the nine Ad7 genotypes defined by restriction endonuclease polymorphisms, we found that the nucleotide sequence of the gp19K gene is very highly conserved, with only a very few changes, none of which resulted in amino acid changes in 16 of the isolates. With isolate 722, there was a single Ala to Val substitution. Gp19K from all the Ad7 isolates examined was functional in the sense that it coimmunoprecipitated with class I antigens; to our knowledge, this is the first demonstration that gp19K from Ad7 clinical isolates binds to class I antigens, and it is in accord with the report that gp19K

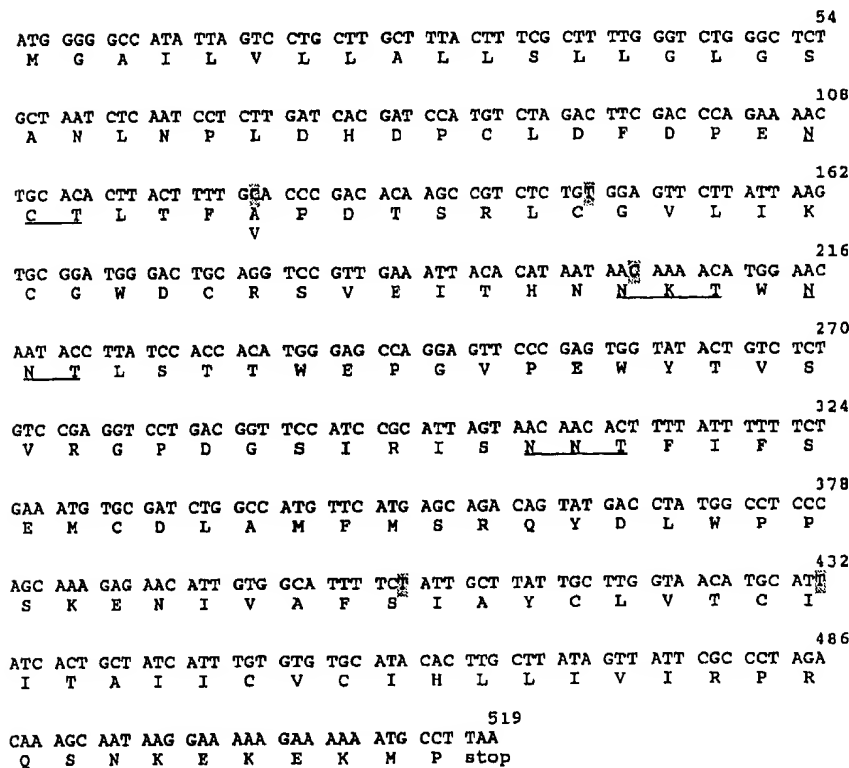


FIG. 1. Nucleotide and amino acid sequence of the E3-gp19K gene and protein from the Ad7 prototype strain, Gomen. Potential glycosylation sites are underlined. Nucleotides (nt) in the clinical isolates that differ from Gomen are identified by the shading. Isolate 722 has a T instead of a C at nt 125, but all other nt are identical to Gomen; the T to C conversion changes the amino acid from Ala to Val. Isolate 641 has a C instead of a T at nt 147, but all other nt are identical to Gomen. All the other isolates have the same sequence as Gomen except that nt 204 is a T instead of a C, nt 405 is a C instead of a T, and nt 432 is a C instead of a T. Except for isolate 722, the amino acid sequence of gp19K from all the isolates is identical to that in Gomen.

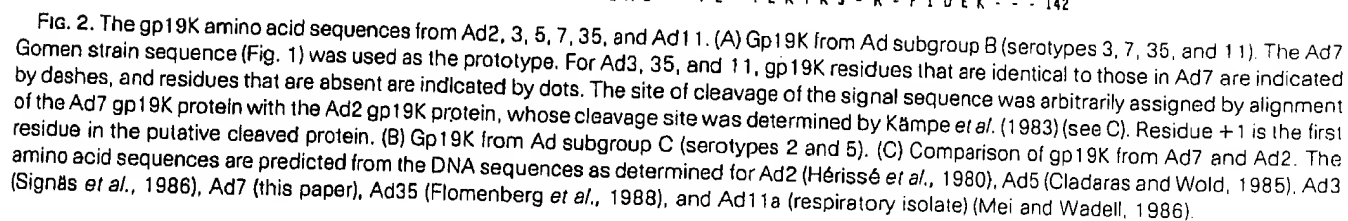
from an Ad35 clinical isolate binds to class I antigens (Flomenberg *et al.*, 1987). Therefore, our data indicate that differences in the sequence of gp19K do not alone account for the different clinical manifestations of the Ad7 clinical isolates. Instead, host and possibly other Ad genes must be the determining factors.

Gp19K is nonessential for Ad replication in cultured cells or in the lungs of hamsters (Morin *et al.*, 1987) or cotton rats (Ginsberg *et al.*, 1989). Although not tested, gp19K is presumably also nonessential for Ad replication during acute infection of humans; if so, then it is particularly surprising that the gp19K sequence is so completely conserved among the Ad7 clinical isolates. Thus, the major implication from our data is that there is very strong evolutionary pressure to maintain the amino acid sequence of gp19K in the clinical isolates of Ad7. Since no other Ad gene has been sequenced from different isolates of the same serotype, we do not know whether our findings are unique to gp19K or are a common feature of Ad genes. Nevertheless, it is interesting to consider why the sequence of gp19K gene is so highly conserved in the Ad7 isolates.

A notable aspect of the Ad2 or Ad5 gp19K proteins is that they bind with different avidities to different class I antigens (see Introduction). Theoretically, if gp19K

binds well to the individual's class I antigens, then an extended acute infection or a persistent infection may result. As a corollary to this hypothesis, different Ad groups or serotypes may have evolved altered versions of gp19K that enable gp19K to bind to class I antigens in different populations. Amino acid sequence comparison of gp19K from different serotypes supports this interpretation. Gp19K from Ad7 is 98% identical to gp19K from Ad3, about 81% identical to gp19K from Ad11, and about 79% identical to gp19K from Ad35 (Fig. 2A). Gp19K of Ad7 and Ad3 are more related to each other than to gp19K from Ad11 and Ad35. Similar results are seen with Ad2 and Ad5 (subgroup C), where the gp19K proteins are 87% identical (Fig. 2B). In contrast, the Ad7 and Ad2 gp19K proteins are only 31% identical over the entire length of the protein (Fig. 2C). Gp19K might be predicted to be polymorphic, based on the reports that gp19K binds to the $\alpha 1$ and $\alpha 2$ domains of class I heavy chain, the polymorphic region of the class I molecule (Burgert and Kvist, 1987; Jeffries and Burgert, 1990).

The sequence comparison between the subgroup B and C serotypes is perhaps more meaningful when we consider only the portion of gp19K that is thought to be most important for binding to class I antigens. As dis-



idues ca. 84–98 in the Ad2 protein), that is conserved among serotypes in different subgroups. There is also a second domain, termed the ER luminal variable domain (residues 1 to ca. 77–83 in the Ad2 protein), that is variable among serotypes in different subgroups. The gp19K proteins of Ad7 and Ad2 are 67% identical

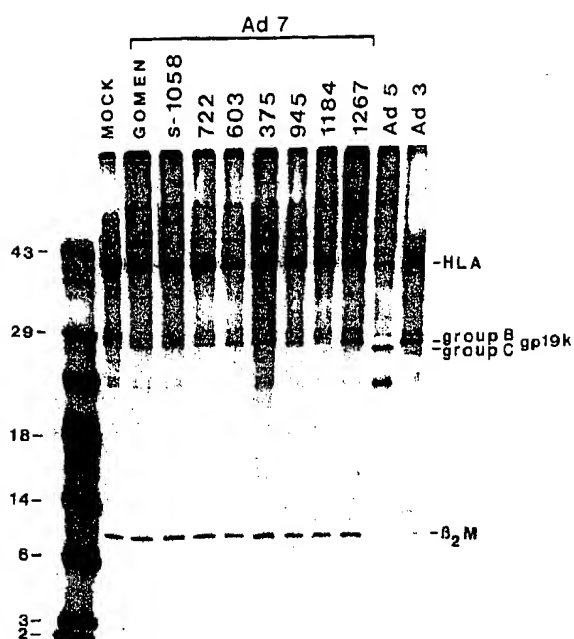


FIG. 3. Immunoprecipitation of MHC class I antigens from human A549 cells infected with representative Ad7 clinical isolates. Class I heavy chains (labeled HLA) were immunoprecipitated using monoclonal antibody W6/32, which is specific to HLA-A, -B, and -C class I antigens. The fully glycosylated gp19K from Ad7 and Ad3 (subgroup B) infected cells that was coimmunoprecipitated is indicated. Also indicated is the coimmunoprecipitated gp19K from Ad5 (subgroup C) (the band labeled "group C" is the fully glycosylated form of gp19K, and the strong faster migrating band is gp19K glycosylated at only one of the two sites). Note that β_2 -microglobulin was also coimmunoprecipitated.

within the ER luminal conserved domain, but only about 25% identical within the ER luminal variable domain (Fig. 2C). Such divergence is not normally observed among Ad genes in different subgroups, and indeed the gp19K gene is among the most diverged of the Ad genes (Chroboczek *et al.*, 1992). The only known function of gp19K from any of the Ad serotypes is the ability to form a complex with class I antigens and retain them in the ER. Since gp19K proteins from subgroup B serotypes are significantly diverged from the subgroup C serotypes, i.e., only 25% identical in the variable domain of gp19K which is essential for binding to class I antigens (Hermiston *et al.*, 1993), it may be that the class I antigens that are bound (with high affinity) by gp19K from subgroup B serotypes are different from those bound (with high affinity) by gp19K from the subgroup C serotypes. If this is so, then this may be the selective pressure that maintains the gp19K amino acid homology within a serotype and a subgroup. In virtually all published experiments where gp19K has been coimmunoprecipitated using antisera to human class I antigens, the W6/32 monoclonal antibody has been used, and this antibody reacts with all human class I antigens. Thus, it is not known to what extent gp19K from different serotypes will bind to different hu-

man class I antigens. Therefore, a detailed investigation is warranted that addresses the binding affinities between the different human class I antigens and gp19K from different serotypes and subgroups.

ACKNOWLEDGMENTS

This work was supported by postdoctoral fellowship F32 CAD8896 to T.W.H. and by Grant R01 CA24710 to W.S.M.W., both from the National Institutes of Health.

REFERENCES

- ADRIAN, T., BEST, B., HIERHOLZER, J. C., and WIGAND, R. (1989a). Molecular epidemiology and restriction site mapping of adenovirus type 3 genome types. *J. Clin. Microbiol.* 27, 1329-1334.
- ADRIAN, T., BECKER, M., HIERHOLZER, J. C., and WIGAND, R. (1989b). Molecular epidemiology and restriction site mapping of adenovirus 7 genome types. *Arch. Virol.* 106, 73-84.
- ANDERSSON, M., PÄÄBO, S., NILSSON, T., and PETERSON, P. A. (1985). Impaired intracellular transport of class I MHC antigens as a possible means for adenoviruses to evade immune surveillance. *Cell* 43, 215-222.
- BERNARDS, R., SCHRIER, P. J., HOUWELING, A., BOS, J. L., and VAN DER EB, A. J. (1983). Tumorigenicity of cells transformed by adenovirus type 12 by evasion of T cell immunity. *Nature* 305, 776-779.
- BURGERT, H.-G., and KVIST, S. (1985). An adenovirus type 2 glycoprotein blocks cell surface expression of human histocompatibility class I antigens. *Cell* 41, 987-997.
- BURGERT, H.-G., and KVIST, S. (1987). The E3/19K protein of adenovirus type 2 binds to the domains of histocompatibility antigens required for CTL recognition. *EMBO J.* 6, 2019-2026.
- CHROBOCZEK, J., BIEBER, F., and JACROT, B. (1992). The sequence of the genome of adenovirus type 5 and its comparison with the genome of adenovirus type 2. *Virology* 186, 280-285.
- GLADARAS, C., and WOLD, W. S. M. (1985). DNA sequence of the E3 transcription unit of adenovirus 5. *Virology* 140, 28-43.
- COX, J. H., BENNINK, J. R., and YEWDELL, J. W. (1991). Retention of adenovirus E19 glycoprotein in the endoplasmic reticulum is essential to its ability to block antigen presentation. *J. Exp. Med.* 174, 1629-1637.
- COX, J. H., YEWDELL, J. W., EISENLOHR, L. C., JOHNSON, P. R., and BENNINK, J. R. (1990). Antigen presentation requires transport of MHC class I molecules from the endoplasmic reticulum. *Science* 247, 715-718.
- FLOMENBERG, P., SZMULEWICZ, J., GUTIERREZ, E., and LUPATKIN, H. (1992). Role of the adenovirus E3-19k conserved region in binding major histocompatibility complex class I molecules. *J. Virol.* 66, 4778-4783.
- FLOMENBERG, P. R., CHEN, M., and HORWITZ, M. S. (1987). Characterization of a major histocompatibility complex class I antigen-binding glycoprotein from adenovirus type 35, a type associated with immunocompromised hosts. *J. Virol.* 61, 3665-3671.
- FLOMENBERG, P. R., CHEN, M., and HORWITZ, M. S. (1988). Sequence and genetic organization of adenovirus type 35 early region 3. *J. Virol.* 62, 4431-4437.
- FOX, J. P., HALL, C. E., and COONEY, M. K. (1977). The Seattle virus watch. VII. Observations of adenovirus infections. *Am. J. Epidemiol.* 105, 362-386.
- GABATHULER, R., and KVIST, S. (1990). The endoplasmic reticulum retention signal of the E3/19K protein of adenovirus type 2 consists of three separate amino acid segments at the carboxy terminus. *J. Cell Biol.* 111, 1803-1810.
- GABATHULER, R., LEVY, F., and KVIST, S. (1990). Requirements for the association of adenovirus type 2 E3/19K wild-type and mutant proteins with HLA antigens. *J. Virol.* 64, 3679-3685.

- GINSBERG, H. S., LUNDHOLM-BEAUCHAMP, U., HORSWOOD, R. L., PERNIS, B., WOLD, W. S. M., CHANOCK, R. M., and PRINCE, G. A. (1989). Role of early region 3 (E3) in pathogenesis of adenovirus disease. *Proc. Natl. Acad. Sci. USA* 86, 3823-3827.
- GOODING, L. R. (1992). Virus proteins that counteract host immune defenses. *Cell* 71, 5-7.
- GOODING, L. R., and WOLD, W. S. M. (1990). Molecular mechanisms by which adenoviruses counteract antiviral immune defenses. *CRC Crit. Rev. Immunol.* 10, 53-71.
- GOODING, L. R., ELMORE, L. W., TOLLEFSON, A. E., BRADY, H. A., and WOLD, W. S. M. (1988). A 14,700 MW protein from the E3 region of adenovirus inhibits cytolysis by tumor necrosis factor. *Cell* 53, 341-346.
- GOODING, L. R., SOFOLA, I. O., TOLLEFSON, A. E., DUERKSEN-HUGHES, P., and WOLD, W. S. M. (1990). The adenovirus E3-14.7K protein is a general inhibitor of tumor necrosis factor-mediated cytolysis. *J. Immunol.* 145, 3080-3086.
- GOODING, L. R., RANHEIM, T. S., TOLLEFSON, A. E., AQUINO, L., DUERKSEN-HUGHES, P., HORTON, T. M., and WOLD, W. S. M. (1991). The 10,400- and 14,500-dalton proteins encoded by region E3 of adenovirus function together to protect many but not all mouse cell lines against lysis by tumor necrosis factor. *J. Virol.* 65, 4114-4122.
- GRAHAM, F. L., SMILEY, J., RUSSELL, W. C., and NAIRN, R. (1977). Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J. Gen. Virol.* 36, 59-72.
- GREEN, M., and WOLD, W. S. M. (1979). "Methods in Enzymology," pp. 425-435. Academic Press, New York.
- GREEN, M., MACKEY, J. K., WOLD, W. S. M., and RIGDEN, P. (1979). Thirty-one human adenovirus serotypes (Ad1-31) form five groups (A-E) based upon DNA genome homologies. *Virology* 93, 481-492.
- HÉRISSE, J., COURTOIS, G., and GALIBERT, F. (1980). Nucleotide sequence of the EcoRI D fragment of adenovirus 2 genome. *Nucleic Acids Res.* 8, 2173-2192.
- HERMISTON, T. W., TRIPP, R., SPARER, T., GOODING, L. R., and WOLD, W. S. M. (1993). Deletion mutation analysis of the adenovirus 2 E3-gp19K protein: Identification of sequences within the endoplasmic reticulum luminal domain that are required for class I antigen binding and protection from antiviral cytotoxic T lymphocytes. *J. Virol.* 67, 5289-5298.
- HIERHOLZER, J. C. (1992). Adenoviruses in the immunocompromised host. *Clin. Microbiol. Rev.* 5, 262-274.
- HORWITZ, M. S. (1990). "Virology," pp. 1723-1740. Raven Press, New York.
- JACKSON, M. R., NILSSON, T., and PETERSON, P. A. (1990). Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum. *EMBO J.* 9, 3153-3162.
- JEFFRIES, W. A., and BURGERT, H.-G. (1990). E3/19K from adenovirus 2 is an immunosubversive protein that binds to a structural motif regulating the intracellular transport of major histocompatibility complex class I proteins. *J. Exp. Med.* 172, 1653-1664.
- KÄMPPE, O., BELLGRAU, D., HAMMERUNG, U., LIND, P., PÄÄBO, S., SEVERINSSON, L., and PETERSON, P. A. (1983). Complex formation of class I transplantation antigens and a viral glycoprotein. *J. Biol. Chem.* 258, 10594-10598.
- KAPOOR, Q. S., WOLD, W. S. M., and CHINNADURAI, G. (1981). A non-essential glycoprotein is coded by early region E3 of adenovirus type 7. *Virology* 112, 780-784.
- KORNFIELD, R., and WOLD, W. S. M. (1981). Structures of the oligosaccharides of the glycoprotein coded by early region E3 of adenovirus 2. *J. Virol.* 40, 440-449.
- KVIST, S., ÖSTBERG, L., PERSSON, H., PHILIPSON, L., and PETERSON, P. A. (1978). Molecular association between transplantation antigens and cell surface antigen in adenovirus-transformed cell line. *Proc. Natl. Acad. Sci. USA* 75, 5674-5678.
- LI, Q.-G., and WADELL, G. (1986). Analysis of 15 different genome types of adenovirus type 7 isolated on five continents. *J. Virol.* 60, 331-335.
- LI, Q.-G., and WADELL, G. (1988). Comparison of 17 different genome types of adenovirus type 3 identified among strains recovered from six continents. *J. Clin. Microbiol.* 26, 1009-1015.
- MEI, Y.-F., and WADELL, G. (1992). The nucleotide sequence of adenovirus type 11 early 3 region: Comparison of genome type Ad11b and Ad11a. *Virology* 191, 125-133.
- MORIN, J. E., LUBECK, M. D., BARTON, J. E., CONLEY, A. J., DAVIS, A. R., and HUNG, P. P. (1987). Recombinant adenovirus induces antibody response to hepatitis B virus surface antigen in hamsters. *Proc. Natl. Acad. Sci. USA* 84, 4626-4630.
- O'DONNELL, B., BELL, E., PAYNE, S. B., MAUTNER, V., and DESSERBERGER, U. (1986). Genome analysis of species 3 adenoviruses isolated during summer outbreaks of conjunctivitis and pharyngoconjunctival fever in Glasgow and London areas in 1981. *J. Med. Virol.* 18, 213-227.
- PÄÄBO, S., BHAT, B. M., WOLD, W. S. M., and PETERSON, P. A. (1987). A short sequence in the COOH-terminus makes an adenovirus membrane glycoprotein a resident of the endoplasmic reticulum. *Cell* 50, 311-317.
- PÄÄBO, S., WEBER, F., NILSSON, T., SCHAFFNER, W., and PETERSON, P. A. (1986a). Structural and functional dissection of an MHC class I antigen-binding adenovirus glycoprotein. *EMBO J.* 5, 1921-1927.
- PÄÄBO, S., NILSSON, T., and PETERSON, P. A. (1986b). Adenoviruses of subgenera B, C, D, and E modulate cell-surface expression of major histocompatibility complex class I antigens. *Proc. Natl. Acad. Sci. USA* 83, 9665-9669.
- PACINI, D. L., DUBOVI, E. J., and CLYDE, W. A., JR. (1984). A new animal model for human respiratory tract disease due to adenovirus. *J. Infect. Dis.* 150, 92-97.
- RAWLE, F. C., TOLLEFSON, A. E., WOLD, W. S. M., and GOODING, L. R. (1989). Mouse anti-adenovirus cytotoxic T lymphocytes. Inhibition of lysis by E3 gp19K but not E3 14.7K. *J. Immunol.* 143, 2031-2037.
- SEVERINSSON, L., MARTENS, I., and PETERSON, P. A. (1986). Differential association between two human MHC class I antigens and an adenoviral glycoprotein. *J. Immunol.* 137, 1003-1009.
- SIGNÄS, C., AKUSJÄRVI, G., and PETERSSON, U. (1986). Region E3 of human adenoviruses: Differences between the oncogenic adenovirus-3 and the non-oncogenic adenovirus-2. *Gene* 50, 173-184.
- SIGNÄS, C., KATZE, M. D., PERSSON, H., and PHILIPSON, L. (1982). An adenovirus glycoprotein binds heavy chains of class I transplantation antigens from man and mouse. *Nature* 299, 175-178.
- TANAKA, Y., and TEVETHIA, S. S. (1988). Differential effect of adenovirus 2 E3/19K glycoprotein on the expression of H-2Kb and H-2Db class I antigens and H-2Kb- and H-2Db-restricted SV40-specific CTL-mediated lysis. *Virology* 165, 357-366.
- WADELL, G. (1984). Molecular epidemiology of adenoviruses. *Curr. Top. Microbiol. Immunol.* 110, 191-220.
- WIGAND, R., and ADRIAN, T. (1986). "Adenovirus DNA. The viral genome and its expression," pp. 409-441. Martinus Nighoff Publishing, Boston, MA.
- WOLD, W. S. M., CLADARAS, C., DEUTSCHER, S. L., and KAPOOR, Q. S. (1985). The 19-kDa glycoprotein coded by region E3 of adenovirus: Purification, characterization, and structural analysis. *J. Biol. Chem.* 260, 2424-2431.
- WOLD, W. S. M., and GOODING, L. R. (1989). Adenovirus region E3 proteins that prevent cytolysis by cytotoxic T cells and tumor necrosis factor. *Mol. Biol. Med.* 6, 433-452.
- WOLD, W. S. M., and GOODING, L. R. (1991). Region E3 of adenovirus: A cassette of genes involved in host immunosurveillance and virus-cell interactions. *Virology* 184, 1-8.